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Antioxidant capacity of medicinal plants from the Province of Córdoba (Argentina) and their in vitro testing in a model food system

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ABSTRACT

The total phenols content (Folin–Ciocalteau assay) and antioxidant capacity (ferric reducing/antioxidant power – FRAP) of 41 plants from Córdoba (Argentina) were analyzed. Phenol content ranged from 8.2 to 100.2 mg GAE/g. FRAP ranged from 85.2 to 1862.0 μmol of Fe(II)/g. *Capparis atamisguea* had the lowest values of total phenols content and antioxidant capacity (8.2 mg GAE/g and 85.2 μmol of Fe(II)/g, respectively), while *Ligaria cuneifolia* exhibited the highest values (100.2 mg GAE/g and 1862.0 μmol of Fe(II)/g, respectively). A significant linear correlation ($p < 0.05$) was found (0.9125) between phenols content and antioxidant capacity. Results support the idea that these plants may be a good source of natural antioxidants for food applications. Plants from the Asteraceae family (the most representative of the Córdoba flora) were further tested for their DPPH radical scavenging activity. Some plant extracts were tested in a simple food system to investigate to their potential use in foods.

1. Introduction

A general recommendation to the public is to increase the intake of foods rich in antioxidant compounds due to their well-known healthy effects. Reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\cdot -}$), hydroxyl radical ($^{\cdot}OH$), and peroxyl radicals ($ROO^{\cdot}$), have been associated with carcinogenesis, coronary heart disease, and many other health issues related to advancing age (Steer, Millgard, Sarabi, Wessby, & Kahan, 2002; Uchida, 2000). Since antioxidants terminate directly ROS radical-mediated oxidative reactions they may be used a method of prevention of aging-associated diseases and health problems. This has led to an accelerated search for antioxidant principles, the identification of natural resources, and the isolation of active antioxidant molecules. Antioxidants have been detected in a number of agricultural and food products including cereals, fruits, vegetables and oil seeds. (Adom, Sorrells, & Liu, 2003; Kalt,

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Forney, Martin, & Prior, 1999; Miller, Rigelhof, Marquat, Prakash, & Kanter, 2000; Naczk & Shahidi, 2006; Netzel, Netzel, Tian, Schwartz, & Konczak, 2007; Parry & Yu, 2004; Yu, Perret, David, Wilson, & Melby, 2002).

In addition to ROS adverse healthy effect, the oxidative deterioration of components in foods is responsible for rancid odors and flavors. These odors and flavors decrease the organoleptic and nutritional quality of processed foods. Antioxidants are required to prevent oxidative reactions during food processing and storage. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG) have been widely used as antioxidants in the food industry (Nawar, 1996). However, the safety of these synthetic antioxidants has been questioned. BHA has shown to be carcinogenic and BHT has been related to internal and external hemorraging at high doses in rats and guinea pigs (Ito et al., 1986). These findings together with consumer interest in natural food additives have reinforced the need for effective antioxidants from natural resources as an alternative to prevent deterioration of foods during processing and storage.

Bioactive phenols are very interesting as antioxidants because of their natural origin and their efficiency as antioxidants (Langley-Evans, 2000; Liu, 2003). Also, the number of publications on the health benefits of polyphenols has increased lately (Ahmad, 1995; Finkel, 2000; Scalbert, Johnson, & Saltmarsh, 2005; Tiwari, 2001). Tea (black and green) is one of the most widely used plant (used as beverage) and rich in polyphenolic compounds collectively known as tea flavonoids (Langley-Evans, 2000; Lie, & Xie, 2000).

The Province of Córdoba is located in Central Argentina. It has an area of 165,321 km^2. It is located between 29°30́–35° SL and 61°47́and 65°46' WL. It is located on a template zone characterized by varied climatic conditions. There are two main geomorphological domains: small mountains (‘‘Sierras”) and flat ‘‘planicies”. The ‘‘Sierras” are small group of mountains located ca. 400 km east of ‘‘Los Andes” (Barboza, Cantero, Nuñez, & Ariza, 2006). The vascular plants of the Córdoba Province include a total of 1958 taxa (Zuloaga, Morrone, & Rodríguez, 1999). The medicinal plants include 669 taxa. Ten families account for more than 50% of all medicinal plants of the Córdoba Province (Barboza et al., 2006). The most represented botanical families of Córdoba medicinal flora are Asteraceae (19.34%), Fabaceae (7.44%), Poaceae (4.16%), and Euphorbiaceae (3.42%) and Solanaceae (3.42%). Cantero and Nuñez (2000) reported the existence of 295 medicinal native species of a total of 373 plant species present in Córdoba Province. In a more recent study, Goleniowsky, Bogiovanni, Palacio, Nuñez, & Cantero, 2006 indicated that the ‘‘Sierra of Comechingones”, a truly geomorphologic–ethnogeographic island located in the ‘‘Sierras of Córdoba”, has 65 families and 149 different genuses of plants that are currently used in traditional medicine with the Asteraceae, Verbenaceae, Fabaceae, and Solanaceae as the most representative families.

Phenolics content of medicinal plants has been determined in many studies (Djeridane et al., 2006; Ivanova, Gerova, Chervenkov, & Yankova, 2005; Katalinic, Milos, Kulisic, & Jukic, 2006; Silva, Souza, Rogez, Rees, & Larondelle, 2007; Wong, Li, Cheng, & Chen, 2006). A review of the literature on plants of Argentina revealed little on the screening of plants for antioxidant activities. To the best of our knowledge we believe there are no previous studies published on antioxidants in medicinal plants of Córdoba. The purpose of this study was to screen the in vitro antioxidant capacity and the total phenols content of medicinal plants collected within the Córdoba Province (Argentina) to find out potential sources of natural antioxidants for food applications. Forty-one plants were collected, washed, and air-dried. Water extracts were prepared to screen for antioxidant activity and phenols content.

2. Material and methods

2.1. Plant material

Samples of 41 medicinal plants were collected within the Province of Córdoba (Argentina). Table 1 shows the scientific, and, when known, traditional uses of such plants. Plants were air-dried (25–30 °C), grounded to fine powder, and stored on tight-seal dark plastic containers until needed.

2.2. Chemicals

All chemical and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St. Luis, MO, USA), Aldrich Chemical Co. (Steineheim, Germany), or Merck (Darmstad, Germany). BHT (2,6-di-*tert*-butyl-4-methylphenol), quercetin, and ascorbic acid were used as standards references. Also, a commercially available *Gingko biloba* extract was analyzed.

2.3. Extract preparation

Plant extracts were prepared according to a standard protocol. To 0.1 g of plant material 50 mL of Milli-Q® deionized water were added. The initial temperature of the water was 95 °C. Infusates were let to stay at room temperature without additional heating. Infusion time was 30 min (final temperatures between 35 and 40 °C). The extracts were filtered and the clear supernatants were used for analytical determinations. Two extracts were prepared for each plant sample.

2.4. Determination of ferric reducing/antioxidant power (FRAP)

The FRAP assay Benzie and Strain (1996) was used with slight modifications. The FRAP reagent was prepared from acetate buffer (pH 3.6), 10 mM 2,4,6-tri(2-pyridyl)-*S*-triazine (TPTZ) solution in 40 mM HCl, and 20 mM iron(III) chloride solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared and warmed to 37 °C in a water bath prior to use. One hundred micro liters of clear filtered extracts were added to 3.0 ml of FRAP reagent. The absorbance of the reaction mixture was recorded at 593 nm after 5 min. The standard curve was prepared accordingly using a stock iron(II) sulphate solution (2000 μM). Results were expressed as μmol of Fe(II)/g dry weight of plant material. All the measurements were taken in triplicate and means and standard deviation values calculated.

2.5. Determination of total phenols content

Total phenols were determined by the Folin–Ciocalteau method (Orthofer & Lamuelas-Raventos, 1999; Singleton & Rossi, 1996). Briefly, two hundred microlitres of extracts were added to 2.5 mL of 1:10 Folin–Ciocalteau reagent (prepared prior to use). After 4 min, 2 mL of sodium carbonate (75 g/L) were added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured. Gallic acid (GA) was used as a standard for determining the phenol content by the Folin–Ciocalteau method. The results were reported in gallic acids equivalents (GAE) per g of sample. The levels of total phenols in these extracts determined according to the Folin–Ciocalteau method are not absolute measurements of the amounts of phenolic materials but are in fact based on their chemical reducing capacity relative to an equivalent reducing capacity of gallic acid. All the measurements were taken in triplicate and means and standard deviation values calculated.

Table 1
Botanical names, families, distribution, and medicinal uses of 41 medicinal plants of the Province of Córdoba (Argentina)

	Distribution[a]	Medicinal uses
Apocynaceae		
Aspidosperma quebracho-blanco Schltdl.	VR	Antidisneic, antiasthmatic, cicatrizant, febrifuge
Mandevilla pentlandiana (A. DC.) Woodson	FA	Drastic
Aristolochacieae		
Aristolochia argentina Griseb.	VR	Antiseptic, diuretic, emenagogue
Asteraceae		
Heterothalamus alienus (Spreng.) Kuntze	AB	Renal affections
Gaillardia megapotamica (Spreng.) Baker var. Megapotamica	AB	Antineuralgic, against headache
Baccharis sessiliflora Vahs. I.	AB	Antiinflammatory
Baccharis stenophylla Ariza	AB	Not reported
Bidens andicola Kunth	AB	Digestive, Antispasmodic
Chrysantemlun tuberculum (Hook et Arn.) Cabrera	AB	Antiseptic, antispasmodic
Eupatorium buniifolium Hook et Arn.	AB	Aperitive tonic
Flaveria haumanii Dimitri et Orfiza	FA	Digestive, emenagoge, dermopatic, febrifuge
Grindelia pulchella Dunal	FA	Antipoison, febrifuge, against rheumatic pains
Microliabum candidum (Griseb.) H. Rob	FA	Not reported
Pterocaulon cordobense Kuntze	AB	Against hepatic affections, pesticide
Thelesperma megapotamicum (Spreng.) Kuntze	AB	Digestive, antispasmodic
Thymophylla pentachaeta var belenidum (D.C.) Strother	FA	Digestive
Trichocline reptans (Wedd.) Rob.	FA	Digestive, aromatizant, diaphoretic
Zexmenia bupthalmiflora (Lorentz) Ariza	FA	Not reported
Bignoniaceae		
Dolichandra cynanchoides Cham.	FA	Anti-diarrheic, antiemetic
Buddlejaceae		
Buddleja mendozensis Benth.	VR	Antiparalytic and muscular tonic
Capparaceae		
Capparis atamisguea Kuntze	FA	Against rheumatic pains, digestive, antiacid
Chenopodiaceae		
Chenopodium mandonii (S. Watson) Aellen	FA	Digestive
Euphorbiaceae		
Acalypha communis Mull. Arg.	FA	Dermopatic
Sebastiana commersoniana (Baill.) L. B. Sm. et Downs	AB	Antiseptic, antifungic
Croton argentinus Mull. Arg.	VR	Digestive
Fabaceae		
Adesmia cordobensis Burkart	NR	Not reported
Astragalus distimens Macloskie	NR	Not reported
Dalea elegans Gill. Ex. Hook. et. Arn. var. Elegans	VR	Not reported
Otholobium higuerilla (Gillies ex Hook.) Grimes	VR	Digestive, vulnerary
Hydrochafitaceae		
Limnobium laevigatum (Humb. & Bonp. Ex Willd.) Heine	NR	Not reported
Lamiaceae		
Lepechinia floribunda (Benth.) Epling	NR	Not reported
Salvia gilliesi Benth.	AB	Febrifuge, against palpitations
Loranthaceae		
Ligaria cuneifolia (Ruiz et Pav.) Tiegh.	AB	Cardiotonic, hypotensive
Malvaceae		
Gaya parviflora (Phil.) Krapov.	NR	Not reported
Passifloraceae		
Passiflora caerulea L.	FA	Tranquilizer, ansiolitic, antispasmodic
Polygonaceae		
Polygonum lapathifolium L.	AB	Emmenagogue, diuretic
Pteromonnina dictyocarpa (Griseb.) B. Eriksen	AB	Anti-diarrheic, digestive
Pteridaceae		
Argyrochosma nivea (Poir.) Windham var. flava (Hook.) Ponce	NR	Diuretic
Rosaceae		
Kageneckia lanceolata Ruiz et Pav.	FA	Febrifuge, emetic
Verbenaceae		
Lantana grisebachii Seckt. Var. Grisebachii	AB	Febrifuge, emetic
Urbania pappigera Phil.		Not reported

[a] AB = abundant, FA = fairly abundant, VR = very rare, NR = not reported.

2.6. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The DPPH radical scavenging activity of plant water extracts was determined by a spectrophotometric method. Briefly, 50 μL of various concentrations of water plant extracts were mixed with 5 mL of a 0.0004% ethanol solution of DPPH. After 16 min the absorbance was read against a blank of at 517 nm. The IC_{50} (inhibitory concentration 50 μg/mL) was calculated as the concentration

of sample necessary to decrease by 50% the initial absorbance of DPPH.

2.7. Antioxidant capacity of plant species of the Asteraceae family in a simple oil system

Of the plant species collected (41), almost 37% (15) of them pertained to the botanical family Asteraceae. Since plants of this family represent a great proportion of the native plants of the Province of Córdoba (Barboza et al., 2006), we evaluated the ability of them to inhibit oxidation by a second antioxidative test (DPPH – 1,1-diphenyl-2-picrylhydrazyl – assay). Also, some of the plant extracts were tested in a simple oil system: fish oil. This way we believe that, in addition to screening the collected plants by means of certain chemical tests, we can better assess the potential of some plants in a system that closely resembles one of a food system or matrix.

2.7.1. Oxidation of fish oil

Fish oil from menhaden (purchased from Sigma Chemical Co. St. Luis, MO, USA), containing approximately 30% omega-3 fatty acids as triacylgycerols, was subjected to an accelerated oxidation procedure according to a method similar to that of Abdalla and Roozen (1999). Fish oil (20 g) containing 0.1% of plant extracts was incubated at 60 °C for 10 consecutive days in darkness.

Water from water extracts was removed by drying in vacuo. Dried plant extracts were added to the container and 20 g of fish oil was added and slightly vortexed. At regular intervals (1, 2, 4, 6, 8, and 10 days) conjugated diene hydroperoxides (CDH) and thiobarbituric acid-reactive substances (TBARS) were measured.

2.7.2. Conjugated diene hydroperoxides (CDH) assay

CDH content was estimated according to the method of Roozen, Frankel, and Kinsella (1994). From each fish oil sample (subjected to accelerated oxidation conditions) 50 mg were mixed with hexane (spectophotometric grade) and vortexed. CDH absorbance was measured at 235 nm.

2.7.3. Thiobarbituric acid-reactive substances (TBARS) assay

TBARS were measured using the method described by Madsen, Sorensen, Skibsted, & Bertelsen, 1998. Briefly, 1 g of fish oil sample was dissolved in 3.5 mL of hexane and 4.5 mL of a TCA (trichloroacetic) and TBA (thiobarbituric acid) mixture (TCA, 7.5%, TBA, 0.35%) was added. After mixing for 5 min in a vortex, the mix was centrifuged at 3000g for 13 min. The TCA–TBA phase was removed and heated in a water boiling bath for 10 min. Absorbance was measured at 532 nm. Results were expressed μmol of malonaldehyde equivalents per kg of fish oil.

3. Results and discussion

3.1. Screening of 41 plant species for antioxidant and total phenol content

The extracts of 41 Córdoba's plants were tested for antioxidant activity using the FRAP assay, which is, according to Benzie, Wai, and Strain (1999) a rapid, reproducible, and easy to perform assay. In this method, the antioxidant activity is determined based on the ability to reduce ferric(III) iron to ferrous (II) iron (Benzie & Strain, 1996). Although this assay was originally developed to measure plasma antioxidant capacity, it can be used to quantify the antioxidant capacity from a wide variety of biological samples from pure compound to fruits, wines, and animal tissues (Katalinic, Milos, Modum, Music, & Boban, 2004). Results were expressed as μmol ferrous iron(II) equivalent per g of sample.

As shown in Table 2, there is a wide range of antioxidant activities within the extracts analyzed, as measured by the FRAP method. The FRAP antioxidant activity ranged from the lowest value of 85.2 (*Capparis atamisguea*) to the highest value of 1862.0 μmol Fe(II)/g (*Ligaria cuneifolia*). Based on the antioxidant data obtained, the medicinal plants were grouped into four categories: low antioxidant power (<100 μmol Fe(II)/g), medium (100–250 μmol Fe(II)/g), high (250–625 μmol Fe(II)/g), extremely high (>625 μmol Fe(II)/g). The number of plants in each of the low, medium, high, and extremely high antioxidant power categories was 3, 14, 15,

Table 2
Antioxidant capacity (determined as FRAP[a]) and total phenols content of 41 medicinal plants of the Province of Córdoba (Argentina)[b]

Botanical name	FRAP (μmol of Fe(II)/g)	Total phenols (mg GAE/g)[c]
Ascorbic acid	12569.5 ± 54.7	–
Quercetin	12613.5 ± 89.2	–
Ginko biloba Extract	9254.2 ± 12.5	–
BHT	1427.8 ± 9.5	–
Ligaria cuneifolia (Ruiz et Pav.) Tiegh.	1861.9 ± 3.7	100.1 ± 0.58
Sebastiana commersoniana (Baill.) L. B. Sm. et Downs	1306.3 ± 3.4	60.0 ± 0.48
Heterothalamus alienus (Spreng.) Kuntze	1073.5 ± 3.5	93.1 ± 1.73
Kageneckia lanceolata Ruiz et Pav.	845.5 ± 21.5	78.9 ± 0.85
Lepechinia floribunda (Benth.) Epling	802.1 ± 2.2	58.6 ± 0.86
Pterocaulon cordobense Kuntze	704.4 ± 8.8	40.2 ± 0.42
Baccharis sessiliflora Vahs. I.	675.9 ± 6.3	48.4 ± 0.87
Thymophylla pentachaeta var. *belenidum* (D.C.) Strother	657.0 ± 26.0	48.3 ± 2.00
Bidens andicola Kunth	643. 0 ± 5.9	54.4 ± 0.08
Microliabum candidum (Griseb.) H. Rob	640.9 ± 36.0	39.5 ± 0.35
Urbania pappigera Phil.	601.1 ± 2.6	30.0 ± 0.85
Mandevilla pentlandiana (A. DC.) Woodson	569.2 ± 16.8	51.2 ± 3.37
Croton argentinus Mull. Arg.	554.6 ± 6.6	50.5 ± 1.14
Thelesperma megapotamicum (Spreng.) Kuntze	553.0 ± 11.3	36.5 ± 0.20
Polygonum lapathifolium L.	470.6 ± 19.5	43.5 ± 1.03
Buddleja mendozensis Benth.	469.6 ± 23.9	40.8 ± 0.42
Argyrochosma nivea (Poir.) Windham var. *flava* (Hook.) Ponce	454.7 ± 7.6	55.2 ± 1.64
Eupatorium buniifolium Hook et Arn.	439.4 ± 9.7	57.6 ± 0.63
Salvia gilliesi Benth.	436.0 ± 2.4	33.2 ± 0.81
Limnobium laevigatum (Humb. & Bonp. Ex Willd.) Heine	404.3 ± 2.2	20.3 ± 1.38
Chrysantemlun tuberculum (Hook et Arn.) Cabrera	372.6 ± 2.0	37.3 ± 1.68
Lantana grisebachii Seckt. Var. *grisebachii*	351.3 ± 15.4	28.2 ± 0.33
Gaya parviflora (Phil.) Krapov.	324.0 ± 5.7	30.2 ± 1.73
Baccharis stenophylla Ariza	313.1 ± 4.2	32.7 ± 1.30
Acalypha communis Mull. Arg.	291.8 ± 14.0	34.2 ± 0.11
Aristolochia argentina Griseb.	265.8 ± 34.9	19.1 ± 1.13
Aspidosperma quebracho-blanco Schltdl.	234.3 ± 12.2	31.1 ± 1.61
Dolichandra cynanchoides Cham.	221.0 ± 11.1	25.1 ± 1.80
Zexmenia bupthalmiflora (Lorentz) Ariza	215.4 ± 1.7	20.1 ± 0.83
Flaveria haumanii Dimitri et Orfiza	179.6 ± 13.1	32.3 ± 0.23
Gaillardia megapotamica (Spreng.) Baker var. *megapotamica*	174.4 ± 1.1	19.9 ± 1.44
Aristolochia argentina Griseb.	172.1 ± 1.8	14.1 ± 1.15
Dalea elegans Gill. Ex. Hook. et. Arn. var. *elegans*	163.5 ± 2.1	15.9 ± 1.43
Trichocline reptans (Wedd.) Rob.	151.9 ± 17.3	11.4 ± 0.04
Astragalus distimens Macloskie	151.8 ± 0.5	12.0 ± 0.06
Adesmia cordobensis Burkart	141.6 ± 6.5	17.4 ± 0.83
Pteromonnina dictyocarpa (Griseb.) B. Eriksen	139.4 ± 5.1	16.3 ± 0.27
Grindelia pulchella Dunal	136.4 ± 4.4	11.3 ± 0.36
Passiflora caerulea L.	122.3 ± 0.6	10.2 ± 0.27
Chenopodium mandonii (S. Watson) Aellen	110.4 ± 1.4	16.2 ± 0.40
Otholobium higuerilla (Gillies ex Hook.) Grimes	86.8 ± 0.4	15.0 ± 0.17
Capparis atamisguea Kuntze	85.1 ± 1.1	8.2 ± 0.99

[a] FRAP – ferric reducing/antioxidant power.
[b] Data expressed as mean ± standard deviation ($n = 3$).
[c] mg GAE/g – milligram gallic acid equivalents per g of sample.

and 9, respectively. Plants with FRAP antioxidant capacity values higher than 1000 μmol of Fe(II)/g were: *L. cuneifolia* (1862.0 μmol of Fe(II)/g), *Sebastiana commersoniana* (1306.3 μmol of Fe(II)/g), and *Heterothalamus alienus* (1073.5 μmol of Fe(II)/g). These plants will probably be further investigated in the future to determine which chemical groups are responsible for such high antioxidant capacity values.

We also determined the FRAP antioxidant capacity for chemical compounds commonly used by the food industry. We quantified FRAP values for BHT, quercetin, and ascorbic acid (Table 2). Both ascorbic acid and BHT are synthetic compounds while quercetin is a natural plant-derived antioxidant. As it can be seen, of all 41 plants analyzed for FRAP, only one (*L. cuneifolia*, FRAP: 1861.9 μmol of Fe(II)/g) had a FRAP value higher than BHT (1427.8 μmol of Fe(II)/g) and another one (*S. commersoniana*, FRAP: 1306 μmol of Fe(II)/g) had a similar FRAP value. The rest of the plant species had lower FRAP values when compared to BHT.

When compared to ascorbic acid and quercetin, FRAP values of the analyzed plant species were roughly from 6.71 to 14.7 times lower than ascorbic acid and quercetin, respectively. However, we should keep in mind that those high FRAP values obtained for ascorbic acid and quercetin are because we performed the assay on highly purified reference standards and not in complex matrices such as that of the plant species analyzed. A commercially available *G. biloba* extract was also tested (Table 2). This extract exhibited a high value of FRAP (9254.2 μmol of Fe(II)/g) when compared to the plant species but lower than FRAP values of BHT, quercetin, and ascorbic acid reference standards.

There was a wide range of total phenols content in the medicinal plants analyzed in this study as it is shown on Table 2. Values of total phenols varied from 8.2 to 100.2 mg GAE/g as measured by the Folin–Ciocalteau method. The medicinal plant with the lowest value of total phenols was *C. atamisguea* while the one with the highest value was *L. cuneifolia*. A significant linear correlation (coefficient "*r*" = 0.9125) between total phenols and antioxidant capacity (FRAP) is shown in Fig. 1. This significant linear correlation between these two variables have been obtained in many studies (Katalinic et al., 2006; Parejo et al., 2003; Silva et al., 2007). Others (Ou, Huang, Hampsch-Woodili, & Flanagan, 2003) have not found a linear correlation between total phenols and antioxidant power. Differences in the response and the degree of the linearity (that is in the "*r*" coefficient) seem to be related to the fact that different authors use different methods for the determination of antioxidant capacity. These methods differ in terms of their assay experimental conditions and principles. In this study we used the FRAP assay because of its simplicity and reproducibility. As mentioned before, we did find a linear correlation between the total phenols and the FRAP level (Fig. 2).

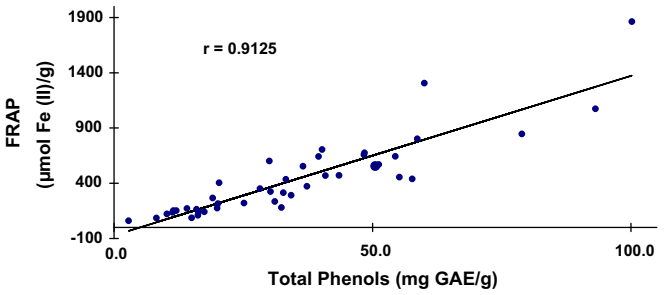

Fig. 1. Linear correlation between the amount of total phenols and antioxidant activity.

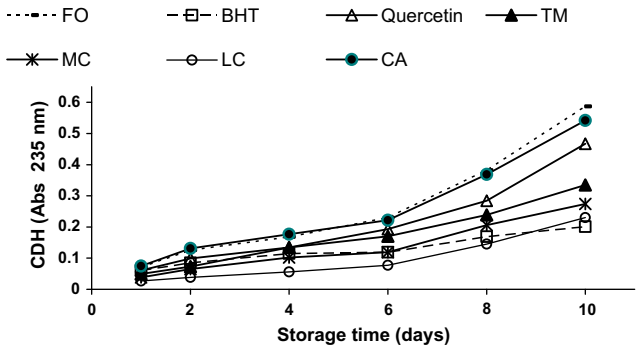

Fig. 2. Conjugated diene hydroperoxides (CDH) formation in fish oil (FO) and fish oil plus BHT, Quercetin, *L. cuneifolia* (LC), *C. atamisguea* (CA), *M. candidum* (MC), and *T. megapotamicum* (TM). BHT and quercetin tested at 0.01%, plant extracts at 0.1%.

3.2. Additional screening of the antioxidant ability of the Asteraceae water plant extracts

Since the majority of the plant species collected in this study pertained to the botanical family Asteraceae, we screened for the antioxidant ability of plant extracts by a second method. Antioxidant ability is a concept that may imply different mechanisms. In the case of the FRAP assay it is the ability to transform Fe(III) to Fe(II). A second method was used further evaluate antioxidant capacity.

The second method used here refers to the free radical scavenging activity of DPPH. Values obtained for this DPPH assay are shown in Table 3 for the 15 Asteraceae plant species. Also, DPPH was quantified for ascorbic acid, BHT, quercetin, and the *G. biloba* extract. As shown in Table 3 the plant extract with the highest DPPH radical scavenging effect was that of *Microliabum candidum* (lowest value of IC_{50}, 198.0 μg/mL) while the plant with the lowest DPPH radical scavenging effect (higher value of IC_{50}, 2009 μg/L) was *Thelesperma megapotamicum*. Between those high and low values a wide range of antioxidant abilities (DPPH) were obtained among the 15 plant species.

The DPPH radical scavenging effects of the standards were all higher than all the 15 Asteraceae plants (Table 3). In fact, all IC_{50}

Table 3
Antioxidative effects (DPPH radical scavenging activity) of water extracts of Asteraceae plant species[a]

Sample	DPPH[b]
Ascorbic acid	11.5 ± 1.3
BHT	15.3 ± 0.7
Quercetin	14.8 ± 0.9
Ginko biloba extract	45.1 ± 1.1
Microliabum candidum (Griseb.) H. Rob.	198.0 ± 3.5
Bidens andicola Kunth var. *decomposita* Kuntze	223.4 ± 5.1
Baccharis stenophylla Ariza	269.3 ± 19.1
Gaillardia megapotamica (Spreng.) Baker var. *megapotamica*	275.7 ± 15.9
Pterocaulon cordobense Kuntze	301.1 ± 10.2
Flaveria haumanii Dimitri & Orfila	308.3 ± 2.2
Eupatorium buniifolium Hook. & Arn.var. *buniifolium*	351,2 ± 6.9
Chrysanthellum indicum DC.ssp. *afroamericanum*	448.7 ± 2.1
Zexmenia buphtalmiflora (Lorentz) Ariza	522.2 ± 37.1
Heterothalamus alienus (Spreng.) Kuntze	559.3 ± 39.1
Thymophylla pentachaeta (DC.) Small var. *belenidium* (DC.) Strother	596.1 ± 15.4
Trichocline reptans (Wedd.) Rob., comb. superfl.	605.8 ± 21.5
Baccharis sessiliflora Vahl	662.3 ± 25.8
Grindelia pulchella Dunal	795.2 ± 11.3
Thelesperma megapotamicum (Spreng.) Herter, comb. superfl	2009.7 ± 84.2

[a] Data expressed as mean ± standard deviation ($n = 3$).
[b] 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity expressed as inhibitory concentration (IC_{50} in μg/mL).

values for BHT (15.3 μg/mL), quercetin (14.8 μg/mL), *G. biloba* (45.1 μg/mL), and ascorbic acid (11.5 μg/mL) were lower than that of the lowest IC_{50} (198.0 μg/mL) for a plant extract. This implies that although there are plants with good antioxidant abilities further concentration and/or purification is needed to achieve better antioxidant capacities.

3.3. Conjugated diene hydroperoxides (CDH) inhibition

Lipid oxidation is a complex set of chemical reactions that leads to the lipid oxidation starts when a free radical is induced to be formed by a metal, exposure to light or decomposition of a hydroperoxide. After initiation, oxidation is propagated via hydrogen subtraction in the vicinity of double bonds. This propagation step implies the formation of isomeric hydroperoxides that frequently carry conjugated diene groups. This is the mechanism of formation of CDH. By measuring CDH formation or inhibition one can assert the effect of different substances on the autoxidation process. From a technological point of view it is important to block the CDH formation initially in order to prevent the subsequent formation of reactive radicals which will undergo further transformation and produce chemical compounds responsible for off-flavours and bad odours.

Fig. 2 shows the effect of some plant extracts on the CDH formation in fish oil. As it can be seen, some extracts and standard references had an inhibitory effect on CDH formation. However, differences in the inhibitory effect were observed among the tested substances and the extracts analyzed. *C. atamisguea* water extract barely had an inhibitory effect on CDH formation being CDH absorbances very similar to that of fish oil alone. *L. cuneifolia* was the water plant extract with the highest inhibitory effect as shown in Fig. 2. BHT and quercetin, as expected, had protective effects on fish oil (inhibition of CDH formation). BHT had a better protective effect than quercetin as evidenced by lower absorbances of CDH at any time during the process. Also, it was observed that quercetin as well as plant extracts may have an inhibitory effect on CDH formation in the first few days of the test (less than 6 days) after which, it seems that the inhibitory effect is lost (8 and 10 days). On the contrary BHT seems to keep at inhibiting CDH formation during the whole oxidation test.

3.4. Formation of thiobarbituric acid-reactive substances (TBARS)

In addition to the CDH, oxidative processes in food can be followed by the amount of TBARS formed. During the propagation phase of the lipid oxidation process oxygen is fixed to the molecule forming hydroperoxides radicals which extract hydrogen atoms from methylene groups rendering hydroperoxides. Low molecular-weight end products, including malonaldehyde, are formed by the oxidative process of polyunsaturated fatty acids. These can be made to react with thiobarbituric acid to produce a reddish compound that can be measured spectrophotometrically.

Inhibition of TBARS production is another way to test the antioxidative effects of natural extracts and chemical compounds. Table 4 shows the TBARS content of fish oil subjected to an accelerated oxidative process with added reference standards (BHT, quercetin) and water extracts from some plants of Córdoba.

As it can be seen from Table 4, for each day of storage (within the same column of Table 4) all samples were statistically different ($p < 0.05$) from the control (fish oil alone). This means that the plant extracts and chemical compound tested had an inhibitory effect on TBARS formation. BHT had the strongest effect on the inhibition of TBARS formation at any time of during fish oil storage since the concentration of TBARS in the fish oil + BHT samples were always the lowest at any time (except for day 2). Quercetin, a natural compound, also had an inhibitory effect of TBARS production but not as strong as BHT.

If we take the concentration of TBARS of fish oil plus BHT at day 10 (Table 4) and compare it with that of fish oil plus TM extract we found that it is very similar implying that the TM extract is capable to prevent fish oil oxidation at a similar extent than BHT. However, we need to keep in mind that the TM extract, as well all other plant extracts, were tested at a concentration 10 times higher (0.1%) than that of BHT (0.01%). If we calculate the number of days needed for samples to reach the TBARS concentration of that of the fish oil control (FO, Table 4) at day 10 of storage, we could estimate a kind of "effectiveness factor" or "protective index" for each of our plant extracts (samples of fish oil alone took 10 days to achieve a TBARS concentration of 42.0 μmol/Kg). Samples of fish oil with BHT (0.01%) would require 2.69 more time to get to the oxidation level of fish oil alone, thus BHT "protects" fish oil by a factor of 2.69. Quercetin, the natural antioxidant tested, would have a protective factor of 1.54 times. The "protective indices" for our plant extracts (tested at 0.1% level) would be: 2.6 for *T. megapotamicum*, 2.42 for *Microliabum candidum*, 2.1 for *L. cuneifolia*, and 1.2 for *C. atamisguea*. So at the plant extract concentration tested (0.1%) some plants (*T. megapotamicum* for example) are almost as good as BHT in preventing fish oil oxidation and some are not (*C. atamisguea*). *T. megapotamicum*, *M. candidum*, and *L. cuneifolia* are even better than quercetin (factor = 1.54) in preventing the oxidation process in fish oil.

As known, tea polyphenols have been studied because of their antioxidant activity (Nihal, Ahmad, Mukhtar, & Wood, 2005). Catechins (monomeric favonols) are present in green tea leaves at levels of 30–40% (Nihal et al., 2005; Wheeler & Wheeler, 2004). The antioxidant ability of tea leaves depend on many factors (tea type, extension of fermentation, etc). However, according to Katalinic et al. (2006) one of the main factors determining the antioxidant capacity of tea leaves is the content of catechins and more specifically of (−)-epigallocatechin-3-gallate (EGCG).

Table 4
Effect of different plant water extracts on the formation of thiobarbituric acid-reactive substances (TBARS) in fish oil during storage (60 °C)[a]

Sample[c]	Thiobarbituric acid-reactive substances (TBARS) (μmol/kg. mean. $n = 3$)[b] Storage time (days)					
	1	2	4	6	8	10
FO	12.1a	17.0a	25.0a	30.2a	35.2a	42.0a
FO + BHT 0.01%	4.3b	8.7b	9.1b	12.8b	14.3b	15.6b
FO + Quercetin 0.01%	8.6c	11.2c	14.9c	19.7c	22.1c	27.1c
FO + TM 0.1%	5.5d	8.3b	12.0d	14.2d	15.8d	16.1d
FO + MC 0.1%	4.9b	8.0b	9.0b	12.5b	15.9d	17.3e
FO + LC 0.1%	4.5b	8.0b	9.5b	13.5e	15.6d	20.0f
FO + CA 0.1%	9.2e	15.5d	23.1e	28.0f	31.2e	35.0g

[a] TBARS values expressed as malonaldehyde equivalents per kg of fish oils. Values within a column followed by different letters are significant different ($p < 0.05$).
[b] Standard deviation values ranged from 0.09 to 0.56; for the sake of clarity SD values were not included within the table.
[c] FO = fish oil; BHT = butylated hydroxytoluene; TM = water extract solids from *T. megapotamicum*; MC = water extract solids from *M. candidum*; LC = water extract solids from *L. cuneifolia*; CA = water extract solids from *C. atamisguea*.

In a recent study, Almajano, Carbó, Lopez Jiménez, & Gordon, 2008 determined the total polyphenol content of green and black tea. They found that the phenolics content of green and black tea was 1844 and 2083 mg of gallic acid equivalents (GAE) per litre of infusion, respectively. Also, the authors evaluated the antioxidant capacities of tea infusions in a model system similar to the one used by us. They found that, on a weight basis, green tea had better antioxidant capacity than black tea. However, due to the fact that they used a different way to measure antioxidant power (TEAC, Trolox equivalent antioxidant capacity) it is not possible to compare the antioxidant capacities obtained by them with tea to the ones measured with our plant extracts.

4. Conclusions

This study supports the idea that some of the medicinal plants studied may be a good source of natural antioxidants to be used by the food industry. In fact, many of the 41 plant species analyzed had high antioxidant capacities as measured in this study. A good correlation between total phenols content and the FRAP values also support the idea that phenols may be the principal contributor of the antioxidant power of botanical materials. Further research is needed (specially on those plants found with very high FRAP and DPPH values) to investigate what chemical compounds are present in the medicinal plants of Córdoba and their potential to be used as possible natural substitutes for artificial antioxidants currently used in food processing. The effect of the use of these natural antioxidants on food sensory properties (such as taste and odour) should be addressed in future research.

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References

Abdalla, A. E., & Roozen, J. P. (1999). Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. *Food Chemistry, 64*, 323–329.

Adom, K. K., Sorrells, M. E., & Liu, R. H. (2003). Phytochemical profiles and antioxidant activity of wheat varieties. *Journal of Agricultural and Food Chemistry, 51*, 7825–7834.

Ahmad, S. (1995). *Oxidative stress and antioxidants defenses in biology*. New York: Chapman and Hall.

Almajano, M. P., Carbó, R., Lopez Jiménez, J. A., & Gordon, H. (2008). Antioxidant and atimicrobial activities. *Food Chemistry, 108*, 55–63.

Barboza, G. E., Cantero, J. J., Nuñez, C. O., & Ariza, L. (2006). Flora medicinal de la Provincia de Córdoba (Argentina). Museo Botánico, Córdoba, Argentina.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Benzie, I. F. F., Wai, Y., & Strain, J. J. (1999). Antioxidant (reducing) efficiency of ascorbate in plasma is not affected by concentration. *Journal of Nutritional Biochemistry, 10*, 146–150.

Cantero, J. J., & Nuñez, C. O. (2000). Las plantas medicinales del sur de la Provincia de Córdoba. Ed. Fundación Universidad Nacional de Río Cuarto. Río Cuarto, Argentina.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry, 97*, 654–660.

Finkel, T. (2000). Oxidants, oxidative stress, and the biology of aging. *Nature (London), 408*, 239–248.

Goleniowsky, M. E., Bogiovanni, G. A., Palacio, L., Nuñez, C. O., & Cantero, J. J. (2006). Medicinal plants from the "Sierra de Comechingones", Argentina. *Journal of Ethnopharmacology, 107*, 324–341.

Ito, N., Hirose, M., Fukushima, H., Tsuda, T., Shirai, T., & Tatenatsu, M. (1986). Studies on antioxidants: Their carcinogenic and modifying effects on chemical carcinogens. *Food and Chemical Toxycology, 24*, 1071–1092.

Ivanova, D., Gerova, D., Chervenkov, T., & Yankova, T. (2005). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology, 96*, 145–150.

Kalt, W., Forney, C., Martin, A., & Prior, R. L. (1999). Antioxidant capacity, vitamin C, phenolics, and anhocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry, 47*, 4638–4644.

Katalinic, V., Milos, M., Kulisic, T., & Jukic, M. (2006). Screening of 70 medicinal plants extracts for antioxidant capacity and total phenolics. *Food Chemistry, 94*, 550–557.

Katalinic, V., Milos, M., Modum, D., Music, I., & Boban, M. (2004). Antioxidant effectiveness of selected wines in comparison with (+)-cathechin. *Food Chemistry, 86*, 593–600.

Langley-Evans, C. (2000). Antioxidant potential of black and green tea determined using the ferric reducing power (FRAP) assay. *International Journal of Food Science and Nutrition, 51*, 181–188.

Lie, C., & Xie, B. (2000). Evaluation of the antioxidant/pro-oxidant effects of tea cathechin oxypolimers. *Journal of Agricultural Food Chemistry, 48*, 6362–6366.

Liu, R. H. (2003). Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. *American Journal of Clinical Nutrition, 78*, 517S–520S.

Madsen, H. L., Sorensen, B., Skibsted, L. H., & Bertelsen, G. (1998). The antioxidative activity of summer savoy (*Stureja hortrensis* L) and rosemary (*Rosamarinus officinalis* L.) in dressing stored exposed to light or in darks. *Food Chemistry, 63*, 173–180.

Miller, H. E., Rigelhof, F., Marquat, L., Prakash, A., & Kanter, M. (2000). Antioxidant content of whole grain, fruits, and vegetables. *Journal of the American College of Nutrition, 19*, 312S–319S.

Naczk, M., & Shahidi, F. (2006). Phenolics in cereals fruits and vegetables: Occurrence, extraction, and analysis. *Journal of Pharmaceutical and Biochemical Analysis, 41*, 1523–1542.

Nawar, W. W. (1996). Lipids. In O. R. Fennema (Ed.), *Food Chemistry* (pp. 225–313). New York: Marcel Dekker Inc.

Netzel, M., Netzel, G., Tian, Q., Schwartz, S., & Konczak, I. (2007). Native austraian foods–a novel source of antioxidants for food. *Innovative Food Science and Emerging Technologies, 8*, 339–346.

Nihal, M., Ahmad, N., Mukhtar, H., & Wood, G. S. (2005). Antiproliferative and proapoptotic effects of (−)-epigallochatechin-3-gallate on human melanoma: Possible implications for the chemoprevention of melanoma. *International Journal of Cancer, 114*(4), 113–521.

Orthofer, R., & Lamuelas-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteau reagents. *Methods in Enzymology, 29*, 152–178.

Ou, B., Huang, D., Hampsch-Woodili, M., & Flanagan, J. A. (2003). When the east meets west: The relation yin–yang and antioxidation–oxidation. *The FASEB Journal, 17*, 127–129.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Saavedra, G., Murcia, M. A., Jiménez, A. M., & Codina, C. (2003). Investigation of Bolivian plant extracts for their radical scavenging activity and antioxidant activity. *Life Sciences, 72*, 1667–1681.

Parry, J., & Yu, L. (2004). Fatty acid content and antioxidant properties of cold-pressed black raspberry seed oil and meal. *Journal of Food Science, 69*, 189–193.

Roozen, J., Frankel, E., & Kinsella, J. (1994). Enzymatic and autoxidation of lipids in low fat foods: Model of linoleic acid in emulsion field hexadecane. *Food Chemistry, 50*, 33–38.

Scalbert, A., Johnson, I., & Saltmarsh, M. (2005). Polyphenols: Antioxidants and beyond. *American Journal of Clinical Nutrition, 81*, 2155–2175.

Silva, E. M., Souza, J. N. S., Rogez, H., Rees, J. F., & Larondelle, Y. (2007). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chemistry, 101*, 1012–1018.

Singleton, V. L., & Rossi, J. A. (1996). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–153.

Steer, P., Millgard, J., Sarabi, D. M., Wessby, B., & Kahan, T. (2002). Cardiac and vascular structure and function are related to lipid peroxidation and metabolism. *Lipids, 37*, 231–236.

Tiwari, A. K. (2001). Imbalance in antioxidant defense and human disease: Multiple approach of natural antioxidant therapy. *Current Science, 8*, 1179–1187.

Uchida, K. (2000). Role of reactive aldehyde in cardiovascular diseases. *Free Radical Biology and Medicine, 28*, 1685–1696.

Wheeler, D. S., & Wheeler, W. J. (2004). The medicinal chemistry of tea. *Drug Development Research, 61*(2), 45–65.

Wong, C., Li, H., Cheng, K., & Chen, F. (2006). A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chemistry, 97*, 705–711.

Yu, L., Perret, J., David, B., Wilson, J., & Melby, C. L. (2002). Antioxidant properties of Cereal Products. *Journal of Food Science, 67*, 2600–2603.

Zuloaga, F. O., Morrone, O., & Rodríguez, D. (1999). Análisis de la biodiversidad en plantas vasculares de la Argentina. *Kurtziana, 27*, 17–167.